TITLE. Effects of simulated weathering on the toxicity of selected crude oils and their components to sea urchin embryos

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ABSTRACT

Artificial weathering of Angolan crude and a Heavy Fuel Oil (HFO) was performed by evaporation and photooxidation. The aliphatic, aromatic, polar and asphaltene fractions of the fresh and weathered oils were isolated. The toxicity of the water accommodated fraction or an oil/fraction dissolved in DMSO was assessed using the sea urchin embryo test. Photooxidation was observed to decrease the aromatics content and increase polar compounds. A slight reduction in the toxicity of Angolan crude was observed following weathering for the water-accommodated fraction and the extract in DMSO, but no effect was seen for the Heavy Fuel Oil. For aliphatic compounds, the toxicity decreased in the order Fresh>Evaporated>Photooxidated for both Angolan crude and HFO. Weathering slightly increased the toxicity of the aromatic and polar fractions of the oil. The aromatic fractions were responsible for most of the toxicity and the polar compounds were the second most important toxic components, despite having less or similar abundance than the aliphatic fraction. The toxic contribution of the aromatic compounds was higher for the HFO than for the Angolan crude. A decrease in the toxicity of Angolan crude following weathering correlated with a reduction in the toxicity of the aliphatic fraction.

KEYWORDS oil spill, weathering, fraction, toxicity, bioassay.

1. Introduction

Petroleum consists of a highly complex mixture of organic compounds, predominantly hydrocarbons and can be characterized by the relative content of the fractions of saturated hydrocarbons, aromatics, resins and asphaltenes (SARA analysis) because its physico-chemical properties are determined by the proportions of these major components. Saturated hydrocarbons (paraffins, iso-paraffins and naphthenes) have low aqueous solubility and toxicity. The aromatic fraction has been identified as containing the compounds mainly responsible for the acute toxicity of petroleum, and polycyclic aromatic hydrocarbons (PAHs) as the family of compounds of greater environmental concern [1].

The European Chemicals Agency (ECHA) recommends the hydrocarbon block method to assess the ecological risk of oil [2]. The method is based on the grouping of known compounds of similar properties into blocks to estimate the predicted environmental concentration (PEC), the Predicted No Effect Concentration (PNEC), and subsequently the PEC/PNEC risk ratio of each individual block and the whole oil. This requires that the properties of individual compounds related to their fate in the environment (solubility, volatility) and toxicity have to be known or estimated. For this reason, it is solely applicable to those compounds that can be resolved by chromatographic techniques (mainly compounds from the saturated and aromatic fractions) but not so for compounds unresolved by gas chromatography such as the unresolved complex mixture (UCM). The proportion of the UCM in a spilt oil is determined by its origin, degree of weathering and/or biodegradation [3]. The UCM of saturated, aromatic and polar fractions have all been shown to be toxic [4-6], so it is important not to ignore unresolved components just because the exact composition cannot be determined by the available analytical methods [7]. The difficulty in establishing causality between composition and effects has already been reported. For example, PAHs may not be the main cause of toxicity for certain oils [8], and the toxic contribution of the polar organic fraction may also be important [7, 9, 10]. Recently Bellas et

al. [11] have shown that the increased toxicity of certain weathered oils cannot be explained on the basis of the changes in total PAH content.

The term "weathering" involves a series of processes (evaporation, dissolution, dispersion, emulsification, photooxidation, biodegradation, spreading, and adsorption) that alter the physical and chemical characteristics of spilled oil. Evaporation is a crucial process in terms of material balance that results in losses of lighter saturate and aromatic compounds (monoaromatic and light PAHs), which is more marked for light crude oils. For this reason, the proportion of PAHs and their toxic contribution increases with evaporation [12]. The photooxidation of aliphatics and aromatics fractions generates more polar and water soluble compounds such as ketones, aldehydes, carboxylic acids and esters [12].

The present study was aimed at better understanding the effects of weathering on oil composition and toxicity. Artificial weathering of oil was performed by evaporation and photooxidation, and chemical fractionation to characterize compositional changes. The toxicity of the selected oils and their fractions were quantified by the sea urchin embryo test.

2. Materials and Methods

The following oils were used: Angolan crude oil (Dalia, API 23.14), Heavy Fuel Oil (HFO, API 11.47) [13].

2.1. Oil weathering

2.1.1. Photooxidation

Photooxidation was performed on the oil samples, to simulate the weathering of oil at sea, using SUNTEST® CPS flatbed xenon exposure system from Atlas (Chicago, USA). It is equipped with 1500B NrB4 Xenon lamp that was operated at a potential of 507.5 W/m^2. An appropriate mass of each

oil sample (ca. 0.4 g) was transferred to pre-weighed petri dishes and spread to obtain uniform layer. Each sample was irradiated for 6 hours.

2.1.2. Evaporation

Evaporation was performed to simulate the weathering of oil over the short-term (ca. 2h) following a spill [14]. Crude oil (1L) was placed in a pre-weighed crystallising dish (2L) in a fume hood (air flow 0.5 m/s) at room temperature for 24h. Evaporative loss was calculated for each oil.

2.2. Coarse fractionation

Open column liquid solid chromatography was used to fractionate the oils. A glass column packed with silica gel (60-100 mesh; 5% H_2O; 40 g) under alumina (grade 1 neutral; 1.5% H_2O; 20 g) was loaded with oil (1 g), and the column eluted with hexane (2 column volumes), dichloromethane (2 column volumes) and methanol (2 column volumes) to provide aliphatic, aromatic and polar fractions respectively. The obtained fractions were used for toxicity tests.

The oil samples were also analysed using thin layer chromatography with flame ionisation detection (TLC-FID) which is well established as an efficient, fast and cost effective method to obtain quantitative data on the composition of oils, more specifically the relative contents of saturates, aromatics, resins and asphaltenes (SARA analysis) [15, 16].

Using a sample spotter SES 3202/IS-02 (Ses GmbH, Nieder-Olm, Germany), 0.8 µL of the DCM oil solution was spotted onto silica-coated quartz rods (ChromaRod®-SIII). A three-step separation was performed using 100% n-hexane to 10 cm, 20:80% n-hexane:toluene to 5 cm and 5:95% methanol:dichloromethane to 2 cm, respectively. All eluents used were analytical grade (Suprasolv grade). After elution, the Chromarods® were dried at 40°C for 5 min to remove solvents and transferred into a MK-5 TLC–FID Iatroscan® apparatus (Iatron Labs, Tokyo, Japan) where each Chromarod® was

scanned with the FID to detect the oil compound classes separated on the silica. The hydrogen flow rate was 160-180 mL min^{-1}, the airflow rate was 2000 mL min^{-1} and the scanning speed was 30 s per Chromarod® burned.

2.3. Preparation of water accommodated fractions and oil extracts

Water accommodated fractions (WAFs) of weathered and unweathered oils (Angolan crude oil and HFO) were prepared by adding 3.5 g of to 87.5 mL of 0.22-µm-filtered seawater (FSW) in a 250 mL bottle with a teflon cap (loading rate, 40 g/L). The mixture was kept in the dark and shaken (150 rpm) for 24 h at 20 ºC. The aqueous phase was separated and used to obtain the FSW dilutions to be tested. The experimental concentrations tested (from 1.25 to 1000 mL/L in geometric increments) were obtained by dilution of the WAF in FSW.

DMSO extraction for each fraction or unweathered/weathered oil (Angolan crude oil and HFO) was performed at a ratio 1:9 (m:m) by orbital shaking (150 rpm) for 16 h at 50^0C and dilutions of the extract in DMSO. For the sea urchin embryo test, 1 mL/L of the extract was added to each vial with FSW. The experimental concentrations tested were 0, 10, 20, 50, 100, 250, 500 and 1000 µL/L.

2.4. Sea urchin embryo test

The sea urchin embryo test was performed in accordance with the method of Saco-Álvarez et al. [17]. Gametes of *Paracentrotus lividus* were obtained by dissection of two of the adults and their maturity (ovum sphericity and sperm mobility) checked with a microscope. The ova were transferred to a 100-mL graduated cylinder containing seawater (5-10 ova/µL), a few drops of sperm (30-100 µL) taken from the male gonad were added through a Pasteur pipette, and the mixture shaken gently to facilitate fertilisation. The fertilisation rate was determined in a Sedgewick-Rafter counting chamber in quadruplicate (n=100), as the proportion of eggs with a fertilisation membrane (> 97%). Within 30 minutes, the fertilised eggs were transferred to vials with 10 mL (WAF) or 4 mL (extracts of oil/fraction

135 in DMSO) of FSW dosed with the oil or fraction to be tested. Each vial received 40 eggs per mL and
136 each dose was performed in quadruplicate.
137
138 The eggs were incubated in the dark at 20 ºC for 48 hours, and the larvae fixed by adding a few drops
139 of 40% formalin. In each vial the maximum length of 35 individuals was measured using an inverted
140 microscope and Leica QWIN image analysis software, version 3.4.0 (Leica Microsystems, Germany).
141 The inhibition of growth in length was quantified as [18]:
142

$$R_i = 1 - \frac{\Delta L_i}{\Delta L_0} \quad (1)$$

143
144
145 where ΔL_0 and ΔL_i are the mean length increases in the control and the ith dose, respectively.
146
147 *2.5. Statistical analyses*
148 The dose-response relationship for each heavy metal was described using the modified Weibull model
149 [19]:
150

$$R = K\left\{1 - \exp\left[-\ln 2\left(\frac{D}{m}\right)^{\alpha}\right]\right\} \quad (2)$$

151
152
153 where R is the response (with K as the maximum value), m is the dose corresponding to the semi-
154 maximum response and α is a shape parameter related to the maximum slope of the response.
155
156 Fitting procedures and initial parametric estimations were performed by minimisation of the sum of
157 quadratic differences between experimental and predicted values using the non-linear least-squares
158 (quasi-Newton) method provided by the 'Solver' macro of the Microsoft Excel spreadsheet. Parametric

estimates were confirmed in the non-linear section of the Statistica 6.0 pack, which was also used to calculate the parametric confidence intervals and model consistency (Student's t and Fisher's F tests, respectively, in both cases with α=0.05).

The EC_{50} of each oil was estimated from the fractions by Concentration Addition (CA) and Independent Action (IA) models. CA model can be formulated as Junghans et al. [20]:

$$\mathrm{EC}x_{mix} = \left(\sum \frac{p_i}{\mathrm{EC}x_i} \right)^{-1} \quad (3)$$

where $\mathrm{EC}x_{mix}$ is the total concentration of a mixture corresponding to the effect x, p_i are the relative proportions of the compounds in a mixture and $\mathrm{EC}x_i$ are the equivalent effect concentration of the single substances.

Independent Action (IA) model [21] is usually written as:

$$E(c_{\mathrm{mix}}) = 1 - \prod_{i=1}^{n} (1 - E(c_i)) \quad (4)$$

where $E(c_{\mathrm{mix}})$ is the total effect corresponding to the mixture and $E(c_i)$ are the effects of the individual constituents.

Toxic units (TU) for each fraction were calculated according to the CA model and the following expression:

$$TU = \frac{p_i ECx_{mix}}{ECx_i} \quad (5)$$

183

184 **3. Results**

185 *3.1. Coarse fractionation*

186 TLC-FID analysis of the weathered samples and their comparison to the original samples shows that
187 the effects of weathering are somewhat different depending on the type of oil (Table 1). Simulated
188 weathering resulted in losses of monoaromatics in the two oils. Aromatics content is decreasing due to
189 photooxidation, while after evaporation in HFO its content is increased (Table 1). The percentage of
190 resins increased following irradiation in all samples due to the photooxidative transformation of
191 aromatics ($p < 0.05$, Table 1), while evaporation caused a slight decrease of the percentage of resins for
192 HFO ($p < 0.05$). Asphaltenes are the most resistant to weathering with both oils showing only slight
193 changes in their content.

194

195 *3.2. Sea urchin embryo test*

196 The WAF of the fresh Angolan crude showed similar toxicity to the evaporated oil suggesting very
197 little change as would be expected 2h post spill. The photooxidised oil was less toxic ($p < 0.05$) (Table 2
198 and Figure 1). The toxicity of the HFO WAFs was remarkably low, and it was not possible to discern a
199 clear trend (Table 2 and Figure 1).

200

201 For the Angolan crude, no significant differences were found between the EC_{50} for the extract of fresh
202 oil and evaporation-weathered oil, although oil weathered by Xenon lamp showed less toxicity than
203 fresh oil ($p < 0.05$) (Table 3 and Figure 2). No significant differences were detected in the EC_{50} for the
204 extracts of fresh and weathered HFO (Table 3 and Figure 2).

205

206 The most evident effect of weathering on oil toxicity was obtained for the aliphatic fraction (Table 3
207 and Figure 2), namely an increase in toxicity parameter values (EC_{10}, EC_{50}, NOEC, LOEC). It can be
208 concluded from the EC_{50} values obtained for this fraction that weathering decreases toxicity ($p<0.05$) in

209 the order fresh>evaporated>photooxidated for both Angolan oil and HFO. The decrease in toxicity was
210 more marked for the medium oil (Angolan crude) than for the heavy oil. Weathering caused an increase
211 in the toxicity (EC_{50} values) of the aromatic fraction, although the differences between fresh and
212 weathered oils were not statistically significant ($p < 0.05$) (Table 3). For this fraction, no significant
213 differences were found between Angolan oil and HFO. The toxicity of the polar fraction was
214 significantly higher for weathered oil than for fresh oil ($p < 0.05$) (Table 3).
215
216 The toxicity of the aromatic and polar fractions were both significantly greater than that of the
217 aliphatic fraction in all cases tested ($p < 0.05$) except for fresh Angolan crude (Table 3 and Figure 2).
218
219 Toxic units were calculated for each fraction according to the concentration addition model, the
220 relative proportions of each fraction in oil and assuming that the toxicity of the asphaltenes was
221 negligible (Table 4). Aromatics explain most of the toxicity and polars are the second most important
222 component, despite having less or similar abundance than aliphatics. The value of toxic units for the
223 aliphatic fraction decreases in the order Fresh<Evaporated<Xenon and increases in reverse order for the
224 polar fraction. The predicted EC_{50} values were calculated based on the toxicity of their fractions and CA
225 and IA models (Table 5).
226
227 **4. Discussion**
228 Aromatic hydrocarbons, as defined by the aromatic fraction, were the main oil components
229 responsible for the toxicity observed for the sea urchin embryo test. A reduction in the toxicity of the
230 aliphatic fraction was observed following weathering and it is probably due to a loss of compounds of
231 low molecular weight. The toxic contribution of this fraction was medium in Angolan crude (0.37-0.03
232 TU) and low (0.07-0.02 TU) for a heavy oil. The toxicity of the aromatic components increased
233 following artificial weathering, in particular for the HFO. The toxic contribution of the polar fraction

234 increases with weathering (Figure 2), and a greater contribution of the polar fraction was observed in the
235 weathered oils compared to the aliphatic fraction (Table 4).
236
237 A slight reduction in the toxicity of the medium Angolan crude (Dalia) was observed following
238 weathering in the sea urchin tests performed with the water-accommodated fraction and the extract in
239 DMSO, but no clear effect of weathering was found for the HFO. Photooxidative treatment with the
240 Xenon lamp produces a slight reduction in toxicity for the Angolan crude in both WAF and extract in
241 DMSO tests, and this correlates with a decrease of the toxic contribution of the aliphatic fraction (Table
242 4). Artificial weathering methods do not substantially change the toxicity of the HFO since the toxic
243 contribution of aromatics is decisive (~0.7-0.8 TU).
244
245 Embryo-larval toxicity obtained for the WAF of a medium oil (Angolan crude) was higher than that
246 of a heavy oil (HFO) (Table 2). However, the toxicity for DMSO extracts of these oils were similar
247 (Table 3), possibly due to the higher capacity and rate of extraction of DMSO compared to water. The
248 low toxicity of the WAF of a HFO is possibly caused by a low transferring rate of hydrocarbons from
249 oil to water and a low concentration of total petroleum hydrocarbons as suggested by Tsvetnenko and
250 Evans [22]. These authors found that: a) the WAF of a heavy oil showed a maximum concentration of
251 total hydrocarbons (TPH) at 600 hours while the maximum TPH for medium and light crude oils were
252 achieved within 24 hours; b) maximum TPH for light and medium crude oils were higher than that of
253 the heavy oil.
254
255 The water accommodated fraction has been considered more suitable for oil toxicity testing than to
256 use a carrier because of a greater resemblance to natural conditions and the impossibility to find a low
257 toxicity solvent capable of dissolving all compounds of oil [23]. The chemical analysis of water-
258 accommodated fraction has raised questions about the class of compounds causing toxicity [8, 24], and
259 these findings underline the need of using other approaches for identification purposes. In this paper,

fractionation and biological testing were used to establish cause-effect relationships between the toxicity of coarse fractions and oil. The use of an extract in DMSO of an oil fraction or oil has made possible to: a) test the potentially active compounds in water [7]; b) consider all the compounds of a fraction irrespective of the proportion that can be solved by chromatographic methods; c) obtain reproducible results with low sample volumes; and d) evaluate the changes of toxicity for a fraction/oil caused by the artificial weathering methods. However, it was not possible to establish causality between the compounds belonging to each fraction and the toxic response; thus, finer fractionation procedures may be recommended in future research.

In the past, similar approaches have been used to assess the contribution of toxic fractions of oil. The purpose of those studies was not to evaluate the effect of weathering on the toxicity of fractions or oil, the fractionation procedures were different, and the results are not fully comparable. Some authors [7, 9] found that aromatic and polar fractions showed significant toxicity in oil and extracts of affected sediments. Other authors [6, 10] performed an extraction and fractionation of the water soluble fraction of a weathered oil and found that the greatest toxic contribution was obtained for the polar fraction. The present paper shows that the polar fraction may play an important role in the effect of weathering on oil toxicity (Figure 2 and Tables 3 and 4). An increase in polars content and a slight decrease in the proportion of total aromatics have been observed in the photooxidative treatment for both Angolan and HFO (Table 1), and this agrees with the results found by Garrett et al [25] for UV treatment of oil. The photooxidation of hydrocarbons is a mechanism that could generate compounds of higher polarity, solubility and reactivity (hydroxylated derivatives, ketones, aldehydes, carboxylic acids and esters) [12] and thus explain the increase of the polar fraction toxicity for the Xenon lamp treatments. However, the authors of the present study have not been able to suggest a process responsible for the increase of toxicity in this fraction for weathered oils by evaporation. Small amounts (1.1-5.1 mg) of the polar fraction were obtained and the relevance of the results suggests performing oil fractionation at a larger scale in future research. Some authors [26] have found that oxygenated hydrocarbons generated by

weathering were eluting in the polar and asphaltene fractions defined by TLC-FID chromatograms, which raises doubts about the assumption of negligible toxicity for the asphaltene fraction.

It is generally accepted that weathering decreases oil toxicity due to the loss of more volatile and soluble compounds [27, 28]. This is moderately in agreement with the results shown for the extracts of a fresh and weathered medium oil but not for the HFO. Neff et al. [24] conclude that monocyclic aromatic hydrocarbons (MAHs) are primarily responsible for the acute toxicity of light oils and toxic contribution of PAHs increases with weathering. In this study, the toxicity of the aromatic fraction to sea urchin embryos slightly vary with weathering for medium and heavy oil (Table 3); hence the decrease of overall toxicity seems to be related to the drop of toxicity of the aliphatic fraction.

The interpretation of the toxicity observed in a sample of water containing hydrocarbons will depend on the available analytical information (resolved plus unresolved) for the compounds of each fraction and the whole set. Oil weathering causes a decrease of the peaks resolved by chromatography and an increase of the UCM which depends on the oil or oil fraction considered [3]. Neff et al. [24] and Barron et al. [8] proposed that other group of components besides MAHs and PAHs might have contributed to the toxicity of the WAF of middle volatility oils; and the polar compounds and UCM were the most likely candidates. Bellas et al. [11] also found that weathering of a standard fuel oil in seawater by magnetic stirring and photooxidation caused an increase of the WAF toxicity with time which was not related to aromatic hydrocarbon levels. The HFO mainly contains a residual fuel and a smaller proportion of lower boiling components; hence the significant presence of the UCM in the chromatograms normally obtained by GC-MS. Some authors [29, 30] have shown that the increase of toxicity with light observed for the WAF of residual fuel oils cannot be explained exclusively by the PAH concentration and suggested that unidentified chemicals, in particular the heterocyclic compounds, could present potent phototoxicity. For these reasons, parental PAHs should not be the only group of compounds monitored after an oil spill.

The predicted EC_{50} for the sea urchin assay by CA and IA models is quite close to the observed values (Tables 3 and 5). This would be in line with the widespread assumption of a non-specific mode of action, called narcosis, for petroleum compounds [31]. Some authors [32] have questioned that the toxicity of PAHs may be due to nonspecific mechanisms. The experimental design of this work is not adequate to discern this, and additivity between compounds and/or petroleum fractions deserves further research.

Conclusions

Weathering decreases the toxicity of the aliphatic fraction and increases the toxicity of the aromatic and especially the polar fractions of the oil. Therefore, the present results stress the role of polar compounds in the toxicity of weathered oils.

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426

427 FIGURE CAPTIONS

428

429 Figure 1. Inhibition of sea urchin larval growth by the water accommodated fraction of Angolan crude

430 oil (up) and HFO (down). The symbols represent fresh oil (—◇—), weathering by evaporation (···□···

431), and weathering by Xenon lamp (- Δ -). Dose in mL/L.

432 Figure 2. Inhibition of sea urchin larval growth by a extract of DMSO of Angolan crude oil (left) and

433 HFO (right). The symbols represent fresh oil or fraction (—◇—), weathering by evaporation (···□···),

434 and weathering by Xenon lamp (- Δ -). The fraction or oil is indicated by text printed on each graph.

435 Dose in μL/L.

436

437 Figure 1

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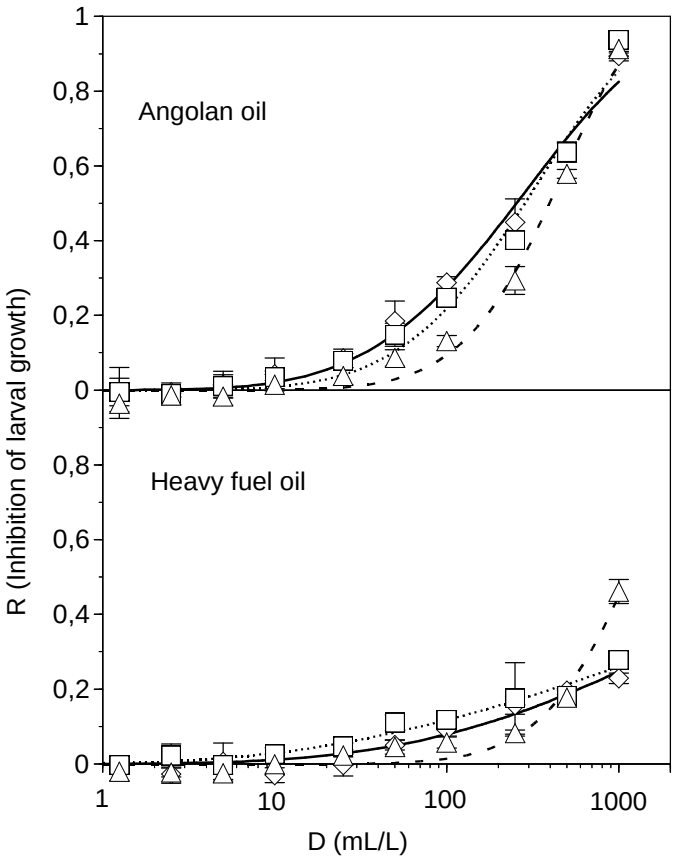

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441 Figure 2

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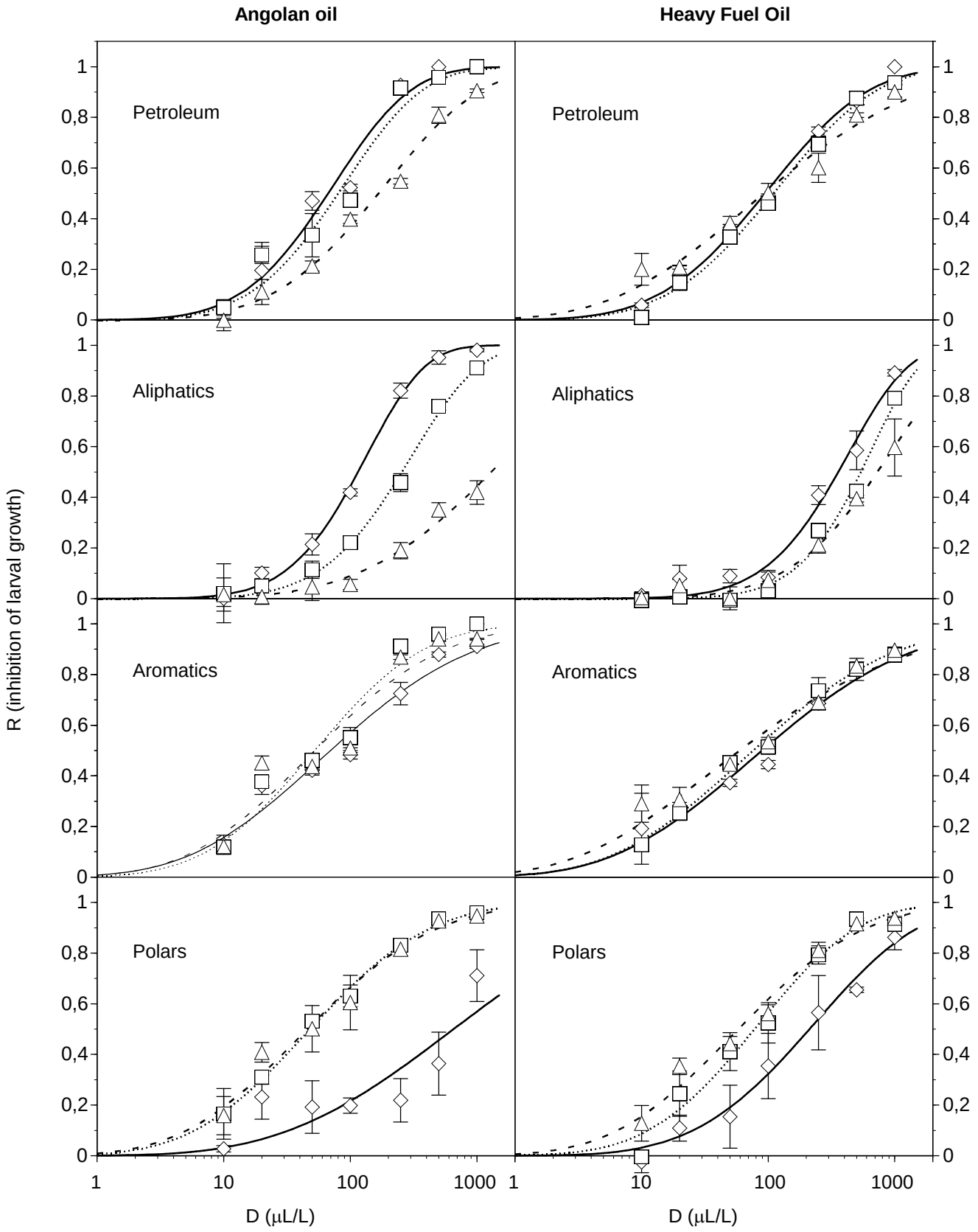

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446 TABLES.

447 Table 1 Aliphatics, monoaromatics, polyaromatics, total aromatics, polars and asphaltene content for
448 fresh and weathered oils. An asterisk indicates a content statistically different from the unweathered
449 fraction (* $p < 0.05$).

450 Table 2. Toxicity thresholds (NOEC. LOEC and EC_{10}) and EC_{50} obtained with sea urchin embryo test
451 for the water accommodated fraction of fresh and weathered Angolan and HFO.

452 Table 3. Toxicity thresholds (NOEC. LOEC and EC_{10}) and EC_{50} obtained with sea urchin embryo test
453 for the extracts in DMSO of fresh and weathered Angolan and HFO and their fractions.

454 Table 4. Toxic units (TU) for the dose corresponding to 50% effect (EC_{50}) calculated according to the
455 concentration addition model for the fresh and weathered oils.

456 Table 5. Predicted EC_{50} values for the DMSO extracts of oil according to concentration addition (CA)
457 and independent action (IA) models applied to the isolated fractions.

458

Table 1

Oil		Content (%) Aliphatics	Monoaromatics	Polyaromatics	Total aromatics	Polars	Asphaltenes
Angolan	No weathering	39.94±8.48	16.04±8.84	23.67±9.37	39.71±5.46	14.51±2.66	5.84±2.17
	Evaporation	41.55±2.23	12.41±2.47	27.64±4.58	40.05±2.20	14.01±2.18	4.39±1.38
	Xenon lamp	37.48±3.41	10.54±2.38	16.91±0.50	27.45±2.32*	30.65±2.32*	4.43±3.55
Heavy fuel	No weathering	16.44±2.70	10.96±8.13	39.22±11.26	50.18±3.27	17.76±2.46	15.63±3.28
	Evaporation	17.16±1.47	10.65±9.27	44.64±12.70	55.28±4.87*	14.93±1.51*	12.63±4.32
	Xenon lamp	17.03±0.85	8.26±0.46	39.30±2.25	47.55±1.95	22.22±1.87*	13.20±1.25

Values ± 95% confidence intervals.

Table 2.

Oil	Weathering	EC_{50} (mL/L)	EC_{10} (mL/L)	NOEC (mL/L)	LOEC (mL/L)
Angolan	No weathering	254.4 (216.7-298.7)	33.4 (24.2-45.9)	10	25
	Evaporation	283.1 (235.1-340.9)	48.5 (32.9-71.5)	10	25
	Xenon Lamp	394.2 (349.4-444.7)	104.0 (78.7-137.3)	25	50
Heavy Fuel	No weathering		147.3 (90.7-238.8)	50	100
	Evaporation		70.3 (47.3-104.4)	25	50
	Xenon Lamp		305.9 (231.3-404.6)	25	50

Values reported with 95% confidence intervals in parentheses.

Table 3

Oil	Oil/Fraction	Weathering	EC_{50} (µL/L)	EC_{10} (µL/L)	NOEC (µL/L)	LOEC (µL/L)
Angolan	Bulk	No weathering	66.8 (50.6-88)	13.2 (7.7-22.2)	10	20
		Evaporation	79.9 (57.1-111.6)	15.2 (8-28.4)	10	20
		Xenon Lamp	166.6 (139.2-199.4)	23.2 (16.4-32.6)	10	20
	Hexane	No weathering	115 (103.8-127.4)	28.9 (23.6-35.3)	10	20
	(aliphatics)	Evaporation	254.6 (230.1-281.7)	52.1 (42.1-64.4)	50	100
		Xenon Lamp	> 1000	112.7 (74.9-169.2)	100	250
	Dichloromethane	No weathering	69.6 (48.1-100.6)	6.2 (3.2-11.3)		10
	(aromatics)	Evaporation	52.1 (35.2-76.8)	7.2 (3.5-14)		10
		Xenon Lamp	52.2 (29.8-91)	5.7 (2-14.1)	10	20
	Methanol	No weathering	654.2 (226.6-1885.1)	32.8 (5.7-170.3)	10	20
	(polars)	Evaporation	47.9 (42.8-53.5)	5.9 (4.8-7.1)		10
		Xenon Lamp	45.7 (34.4-60.6)	4.8 (2.9-7.6)		10
Heavy Fuel	Bulk	No weathering	94.5 (81.3-109.8)	13.6 (10.4-17.8)		10
		Evaporation	109.8 (95.8-125.7)	15.8 (12.3-20.2)	10	20
		Xenon Lamp	93.9 (69.6-126.4)	7.3 (4.3-12)		10
	Hexane	No weathering	358.1 (294.7-435)	80.9 (52.1-125.5)	20	50
	(aliphatics)	Evaporation	537.8 (476.9-606.4)	145 (108.4-193.9)	100	250
		Xenon Lamp	727.5 (645.6-819.8)	125.3 (96.2-163.1)	100	250
	Dichloromethane	No weathering	89.4 (67.3-118.5)	7 (4.2-11.3)		10
	(aromatics)	Evaporation	75.8 (65.2-88.2)	6.7 (5.1-8.6)		10
		Xenon Lamp	59.3 (42.8-82.1)	3.9 (2.2-6.5)		10
	Methanol	No weathering	212.2 (170.4-264.1)	25.6 (16.6-39.1)	50	100
	(polars)	Evaporation	77.9 (58.5-103.5)	11.2 (6.7-18.5)	10	20
		Xenon Lamp	57.9 (44.2-75.6)	6.4 (4-10)	10	20

Values reported with 95% confidence intervals in parentheses.

Table 4

Oil	Weathering	Fraction	TU
Angolan	Fresh	Aliphatics	0.37
		Aromatics	0.61
		Polars	0.02
		Asphaltenes	0
	Evaporation	Aliphatics	0.13
		Aromatics	0.63
		Polars	0.24
		Asphaltenes	0
	Xenon lamp	Aliphatics	0.03
		Aromatics	0.43
		Polars	0.54
		Asphaltenes	0
Heavy fuel	Fresh	Aliphatics	0.07
		Aromatics	0.81
		Polars	0.12
		Asphaltenes	0
	Evaporation	Aliphatics	0.03
		Aromatics	0.77
		Polars	0.20
		Asphaltenes	0
	Xenon lamp	Aliphatics	0.02
		Aromatics	0.66
		Polars	0.32
		Asphaltenes	0

Table 5

Oil	Weathering	Predicted $EC50_{oil}$ (μL/L)	
		CA	IA
Angolan	Fresh	106.4	96.5
	Evaporation	81.6	74.8
	Xenon Lamp	80.8	61.8
Heavy fuel	Fresh	144.7	131.7
	Evaporation	104.9	93.9
	Xenon Lamp	82.7	59.1